SUPPLEMENTAL ONLINE MATERIAL

Supplemental Figure Legends

Figure S1. Anterior (A) or posterior (B) midguts from animals fed a lipid-depleted diet supplemented with 1X cholesterol (1X C). DAPI (blue), Prospero (red). C) cholesterol or cholesterol ester per 25 midguts (mean +/- SD) for animals raised on the indicated diets. * = p < 0.05, ** = p < 0.01 (t-test). D) % ee cells relative to total posterior cells for animals fed a control diet for ten days after eclosion, and then switched to the indicated diets for ten days. Circles show individual values color-coded for diet: control diet (CD, black), lipid-depleted diet (LD, red), 1X cholesterol diet (1X C, green), 4X cholesterol (4X C, blue). Bar = Average value. N = 15 for all samples. (E-H). Posterior midguts from animals fed on diets supplemented with fatty acid (E); triglyceride (F); 1x ergosterol (G), 4x ergosterol (H). DAPI (blue); Prospero (red); Scale bar =40 μm. (I) % enteroendocrine (ee) cells relative to total posterior cells for animals raised on the indicated diets (E-H). Circles show individual values color-coded for diet as indicated (N = 15 for all samples). Bar = Average value; *** = p < 0.005 (t-test). J) Average gene expression values for the indicated NPC genes from the triplicate RNAseq from posterior (y-axis) vs anterior midgut (x-axis) showing higher expression of most in posterior. (K-L) Number of phospho-histone H3 (PH3) positive cells (K) or TUNEL positive (L) cells per posterior midgut from animals raised on the indicated diets. Triangles show individual values color-coded for diet as indicated (N = 18 for all samples). Bar = Average value; *** = p < 0.005, n.s. = not significant, (t-test).

Figure S2. Hr96 affects the production of ee cells in the anterior (A-D) and middle (E-H) midguts from animals of the indicated genotypes: (A,E) *Hr96*$^{1/+}$ (control); (B,F) *Hr96*$^{1/1}$; (C,G)

Hr96²ˣ. (D,H) % ee cells relative to total anterior (D) or middle cells (H) for animals of the indicated genotypes. Individual values are shape-coded by genotype (N = 10 for all samples). Bar = Average value; *** = $p < 0.005$ (t-test). (I-K) Posterior midguts from animals raised on a control diet in which myo1A-GAL4 alone (control) (I) or myo1A-GAL4::UAS-Hr96 (J) were expressed in enterocytes. (K). % ee cells for animals shown in I, J. (N = 10 for all samples). Bar = Average value; *** = $p < 0.005$ (t-test). (L-O) Posterior midguts of control diet raised animals expressing either Npc2b-RNAi (L,N) or ACAT-RNAi (M) driven by either act-GAL4 (L,M) or esg-GAL4 (N,O). (P) qPCR verification that the RNAi lines (color coded as in Fig. 2H,I) reduce midgut RNA levels. DAPI (blue); Prospero (red); Scale bar =40 μm. Q) The relative expression based on triplicate RNAseq from posterior midguts of $Hr96^{1/1}$ compared to controls ($Hr96^{+/1}$) of a panel of genes known to function genetically in Notch signaling. * = Significant increases compared to control (t-test).

Figure S3. (A-C) Posterior midguts stained for Delta (white) from wild type animals raised on the indicated diets: (A) control; (B) lipid-depleted; (C) 4X cholesterol; DAPI (blue); A'-C' (Delta channel alone). Scale bar =40 μm. D) Delta fluorescence per cell from the animals in A-C. Circles show individual values color-coded for diet as indicated (N = 20 for all samples). Bar = Average value; ** = $p < 0.01$, **** = $p < 0.005$ (t-test). (E-H) Posterior midguts stained for Delta (white) from animals with RNAi-induced knockdown of Npc2b or ACAT in enterocytes and raised on control diets: (E) act-GAL4 control; (F) NPC2b-RNAi 1; (G) NPC2b-RNAi2; (H) ACAT-RNAi; DAPI (blue); Delta (white); E'-H' (Delta channel alone). Scale bar =40 μm. arrows: A, C, E, H (ISCs); B, F,G (ISCs and downstream cells). I) Delta fluorescence per cell from the animals in E-H. Circles show individual values color-coded for diet as indicated (N =

20 for all samples). Bar = Average value; ** = p < 0.01, **** = p < 0.005 (t-test). Control (J) and Hr96[1] homozygous (K) MARCM clone (stained for Delta (J',K') and clonal marker (green). arrowhead (J,J') shows and ISC-EB pair with little Delta in EB; arrowhead in (K,K') shows a 2-cell clone with increased and persistent Delta staining. L) Quantitation of the increased frequency of cell pairs in Hr96 mutant clones. M) Delta fluorescence per cell in controls vs Hr96 clones from J,K.

Figure S4. Co-localization of Delta and Rab7 in midgut enterocytes from animals cultured on different diets. Midguts stained with Delta-GFP and Rab7 from animals (A) on a control diet (CD) and B) on a lipid-depleted diet (LD). C) %of cellular vesicles with Delta and Rab7 co-localized in enterocytes from animals on a control diet (CD) or a lipid-depleted diet (LD). **** = p < 0.005 (t-test).

Figure S5. (A,B) Ovariole containing several ovarian follicles stained for Delta from control (A) or Npc2b-RNAi (B). (see quantitation in Fig. 5E.) (C,D). Ovariole containing several ovarian follicles stained for NECD from control (C) or Npc2b-RNAi (D). General and membrane staining is strongly increased. (E-H) Follicle cells stained with DAPI from an $Hr96^{1/+}$ (control) (E,G) or $Hr96^{1/1}$ (F,H) stage 10 (E,F) or stage 14 (G,H) follicle. Yellow arrows in (H) indicate excess nuclei that have degenerated. (I) The average number of follicle cells per visible side is greater in $Hr96^{1/1}$ compared to control. ** p<0.01 t-test. (J) % of ovarioles with PH3 staining is increased in Hr96[1/1] mutants. ** p<0.01 t-test. (K,L) (M,N). Ovariole containing several ovarian follicles stained for Hindsight (red) from $Hr96^{1/+}$ (control) (K) or $Hr96^{1/1}$ (L). DAPI (blue). Scale bar = 50μm. Similar levels of Sec61 expression in HEC116 cells treated with

vehicle (M) or Simvastatin (N). Western blot assaying Delta-like ligand 4 (DLL4) levels vs

tubulin in HCT116 cells treated with vehicle or Simvastatin. (P) Quantitation of O showing a

significant increaseDLL4 expression relative to tubulin. (bars = average +/- SD) * = P<0.05 (t-

test). Q. DLL4 staining relative to tubulin in HEK293 cells treated with vehicle alone (Veh.) or

LXR agonist. Lanes are from independent assays. R) Quantitation of Q showing that average

DLL4 levels relative tot tubulin decreased significantly (bars = average +/- SD) ** = P<0.01 (t-

test).

Figure S6. Effect of diet on enteroendocrine tumor growth. A) Enteroendocrine tumors

induced by expressing Notch RNAi (N-RNAi) were quantitated in 2,500 μm^2 zones as described

in the text, for animals grown on the indicated diets: control diet (CD), LD (lipid-depleted diet),

at 4 days, or 9 days after tumor induction. B) The number of cells in clonal tumors of either

controls ($N^{+/-}$) or homozygous mutants ($N^{-/-}$) from animals grown on the indicated diets. 4XC

(4x cholesterol supplemented diet).
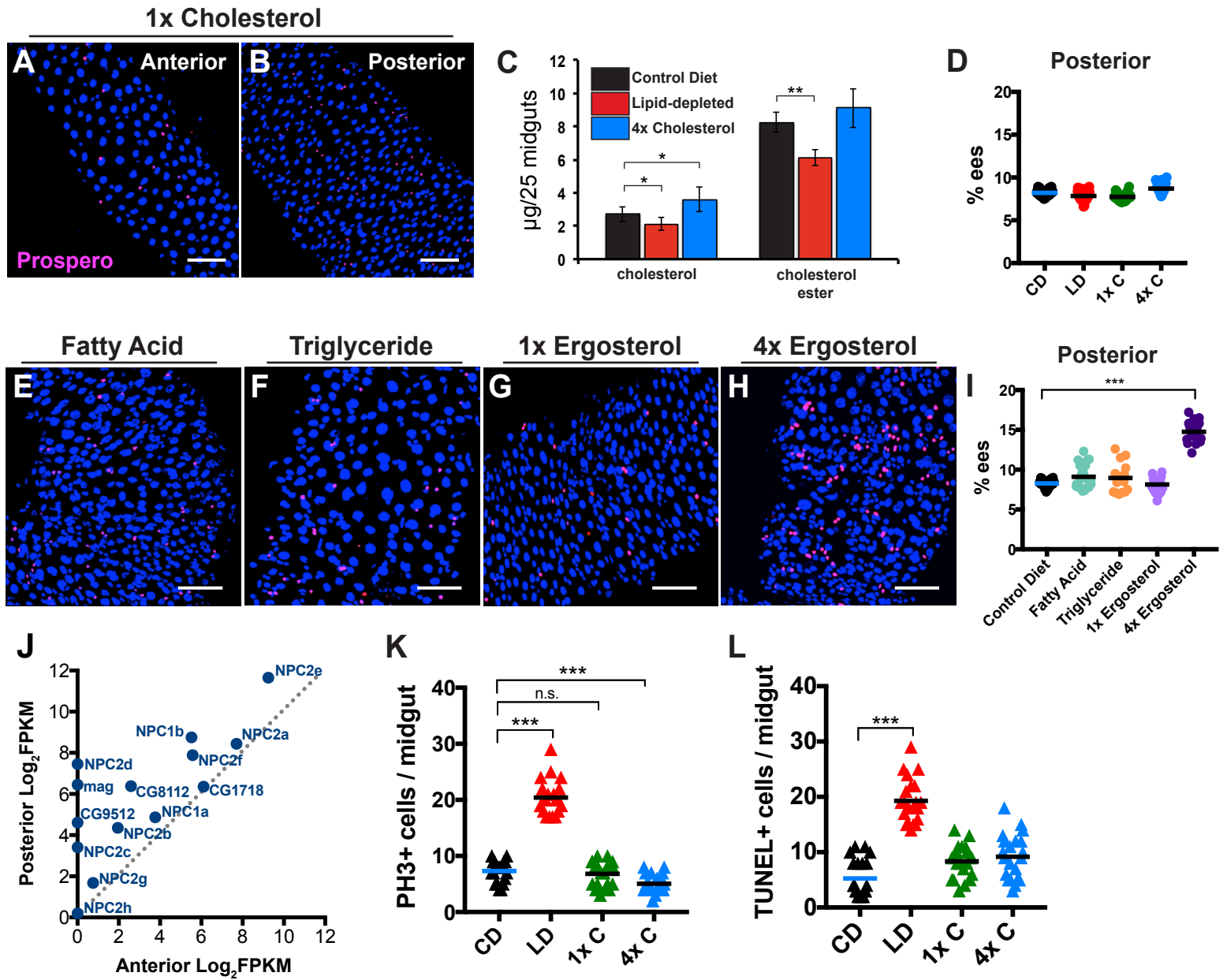
Figure S1. Related to Figure 1
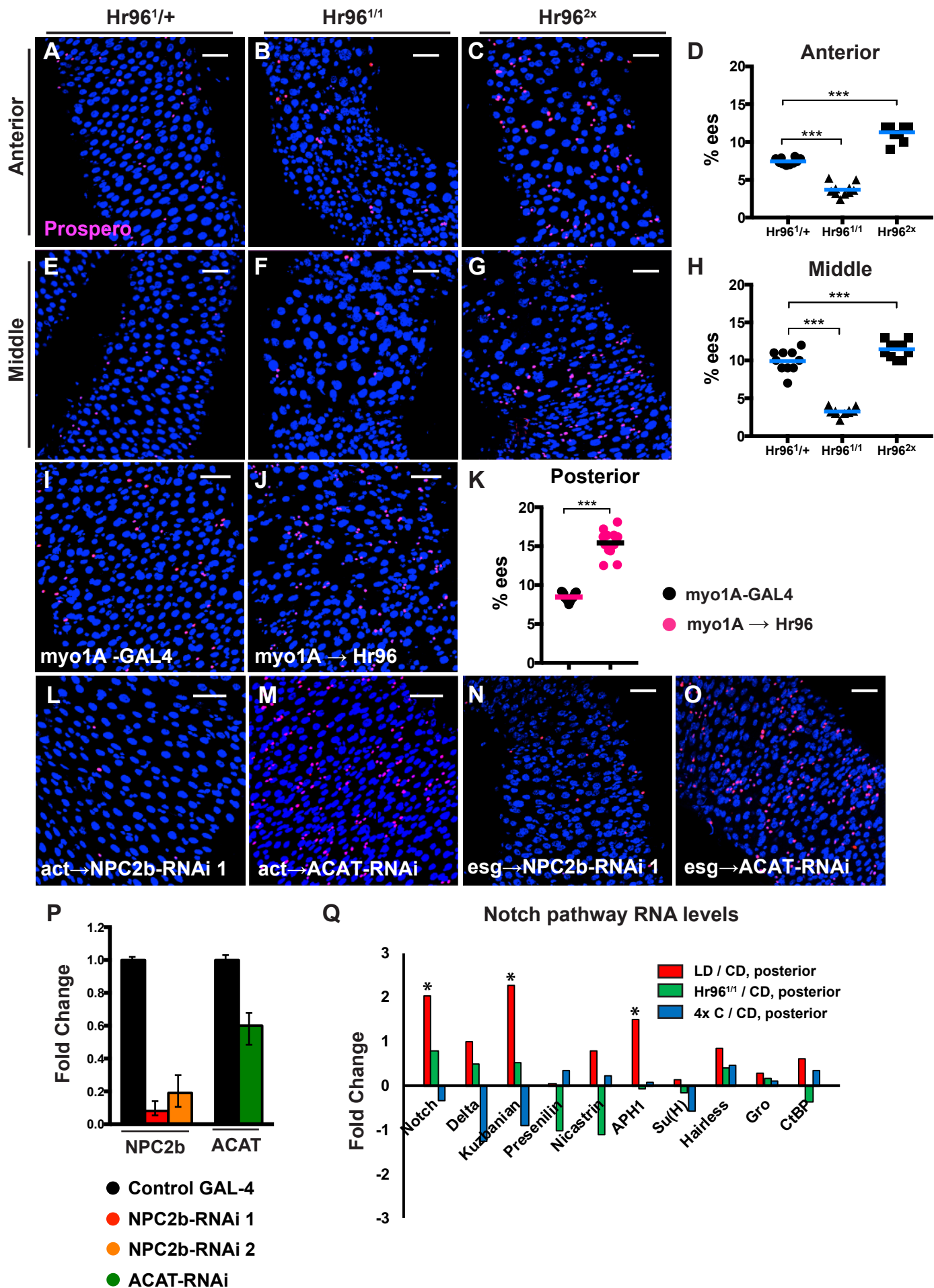
Figure S2. Related to Figure 2
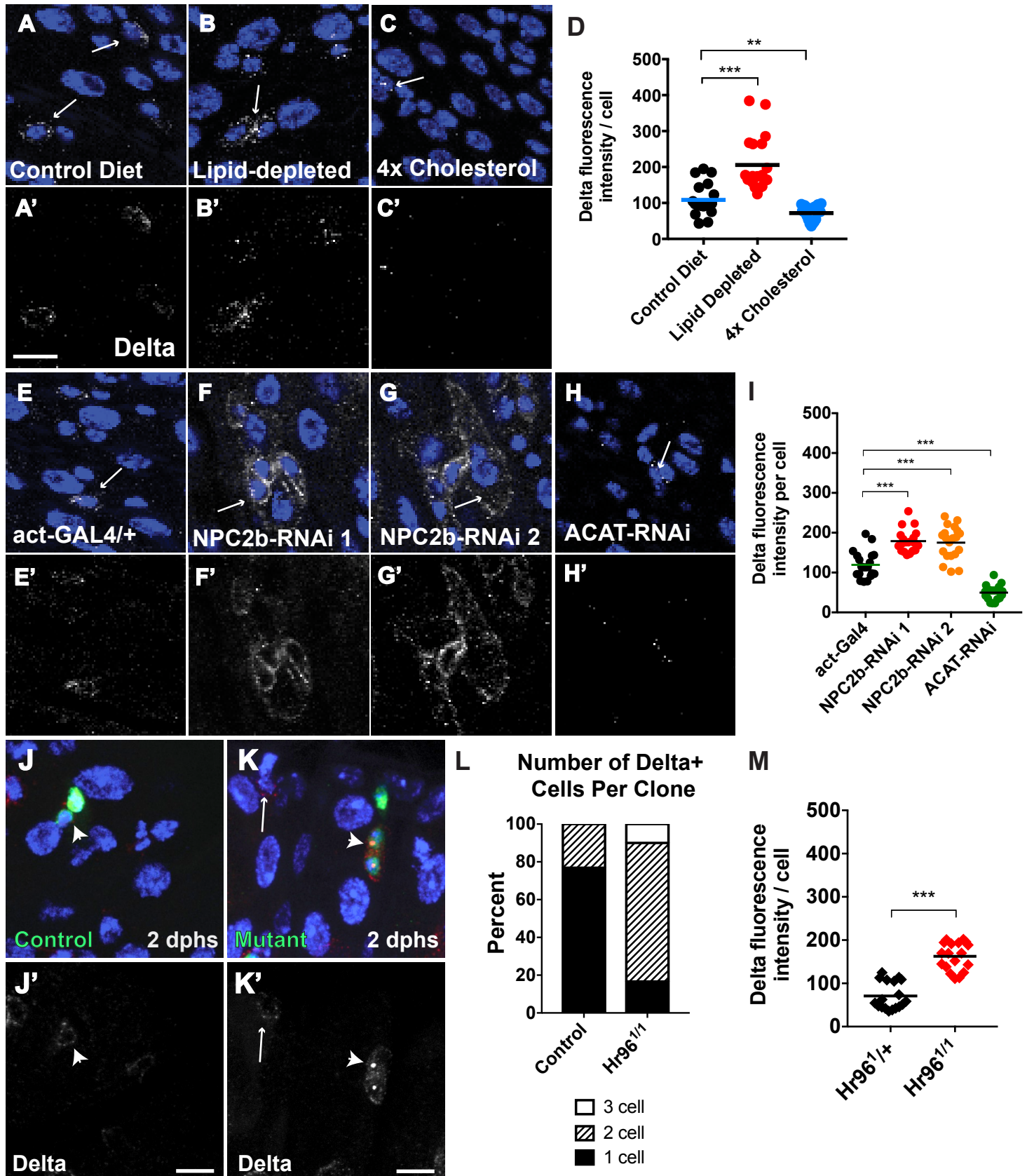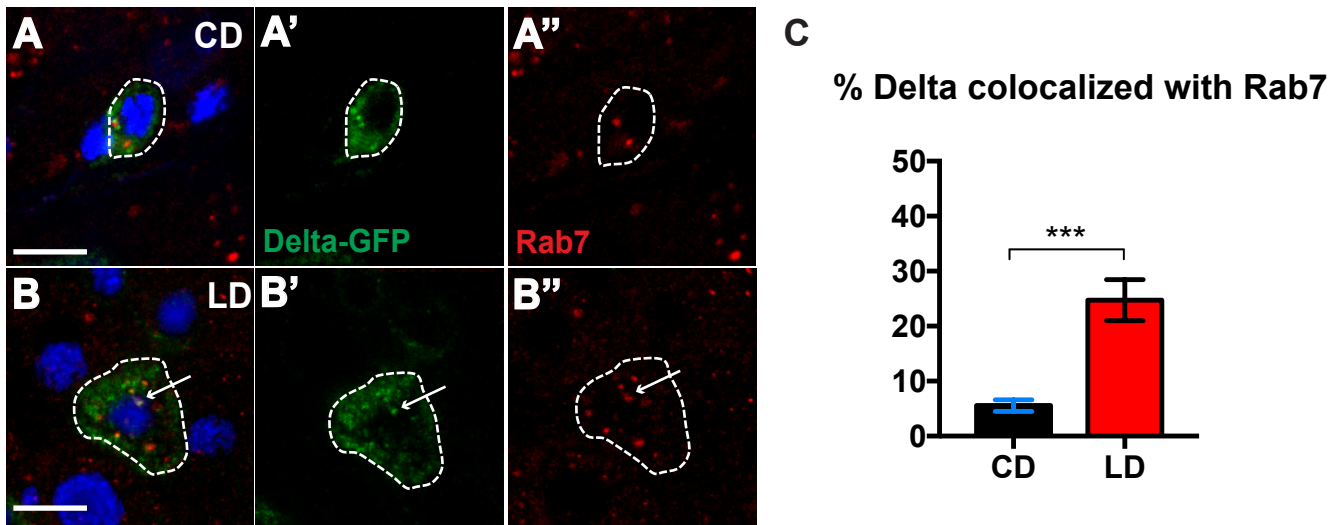
Figure S3. Related to Figure 3

A CD
A' Delta-GFP
A'' Rab7
B LD
B'
B''

C

% Delta colocalized with Rab7